17-β-Estradiol Increases Expression of 52-kDa and 60-kDa SS-A/Ro Autoantigens in Human Keratinocytes and Breast Cancer Cell Line MCF-7

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SS-A/Ro autoantibodies are detected in high frequency in patients with subacute cutaneous lupus, neonatal lupus, systemic lupus erythematosus, and Sjögren syndrome. It has been reported that estrogen was capable of inducing cell surface expression of SS-A/Ro antigens in human keratinocytes, although the molecular forms of the antigen or antigens were not defined. In this study, we analyzed the effects of estrogen on cultured cells by reverse transcriptase polymerase chain reaction and Western blot analysis with respect to both 52-kDa and 60-kDa SS-A/Ro autoantigens. At concentrations of 10^{-8} to 10^{-7} M, 17-β-estradiol induced up to a 5-fold increase of both 52-kDa and 60-kDa SS-A/Ro mRNA in human keratinocytes compared with untreated cells. Hormonal depletion of the human breast cancer cell line MCF-7 showed decreased levels of SS-A/Ro mRNA and protein, and the addition of estradiol led to an increase in SS-A/Ro expression. The estrogenic effect might be mediated through estrogen receptor and the putative estrogen response element at the 5′ region of both SS-A/Ro genes. If the production of autoantibodies is governed at least in part by an antigen-driven process, as has been proposed, our results linking the expression of both forms of SS-A/Ro proteins to estrogenic stimulation may help to explain the high frequency of anti-SS-A/Ro autoantibodies observed in diseases affecting predominantly females. ***Key words: estrogen/autoantibody/gene expression.***
J Invest Dermatol 107:610–614, 1996

Autoantibodies to SS-A/Ro are often found in the sera of patients with systemic lupus erythematosus (SLE), Sjögren syndrome, subacute cutaneous lupus erythematosus, and neonatal lupus erythematosus (for reviews, see McCauliffe and Sontheimer, 1993; Chan and Buyon, 1994; Reichlin, 1995). Because SLE and Sjögren syndrome are predominant in women, some sex-related factors may be involved in the pathogenesis of these autoimmune diseases and also in the production of autoantibodies. There is clinical and experimental evidence indicating that abnormal estrogen metabolism may exist in some autoimmune diseases, including SLE and Sjögren syndrome (Lahita *et al*, 1979, 1982). For example, the production of autoantibodies, including antibodies to cardiolipin, was induced in normal mice by administration of exogenous estrogen (Ansar Ahmed *et al*, 1989; Ansar Ahmed and Verthelyi, 1993). In contrast, famoxifen, an estrogen antagonist, had a beneficial therapeutic effect on the development and course of murine experimental SLE (Sthoeger *et al*, 1994). In 1988, Furukawa *et al* showed enhanced binding of anti-SS-A/Ro and anti-SS-B/La autoantibodies to cultured human keratinocytes treated with estradiol. Their results implied that estradiol increased the synthesis of these autoantigens, which were then expressed on the cell surface of keratinocytes and subsequently became more accessible to autoantibodies.

It has been shown that human sera containing anti-SS-A/Ro antibodies recognize two proteins of 52 and 60 kDa (Ben-Chetrit *et al*, 1988; Rader *et al*, 1989). The 60-kDa protein is bound to small cytoplasmic RNAs known as hY1, hY3, hY4, and hY5 RNAs as a subfamily of low abundant ribonucleoprotein particles (Wolin and Steitz, 1984). Molecular cloning of the 60-kDa protein (Deutscher *et al*, 1988; Ben-Chetrit *et al*, 1989) and the 52-kDa protein (Chan *et al*, 1991; Itoh *et al*, 1991) has shown conclusively that these are distinct proteins with no apparent regions of similarity in amino acid sequence. The 60-kDa protein can be reconstituted with hY RNAs to form ribonucleoprotein complex *in vitro* (Deutscher *et al*, 1988), but the putative interaction of the 52-kDa protein with the hY ribonucleoproteins has not been demonstrated.

In this study, *in vitro* experiments were designed to determine the effects of estrogen on cultured human keratinocytes and on estrogen receptor–positive cancer cell line MCF-7 with respect to the expression of both 52-kDa and 60-kDa SS-A/Ro proteins. The estrogenic influence on the expression of SS-A/Ro and the possible relation to autoantibody production are discussed.

MATERIALS AND METHODS

Cell Culture Second to fourth passages of human keratinocytes from neonatal foreskins (Clonetics, San Diego, CA) were grown in serum-free keratinocyte growth medium (KGM; Clonetics) containing 30 μg bovine pituitary extract per ml, 0.1 ng human epidermal growth factor per ml, 5.0 μg insulin per ml, 0.5 μg hydrocortisone per ml, 50 μg gentamicin per ml, and 50 ng amphotericin-B per ml. Calcium chloride concentration was adjusted to 0.1 mM. Human breast cancer cell line MCF-7 (American Type Culture Collection, Rockville, MD) was grown in RPMI-1640 medium

Manuscript received February 8, 1996; revised April 26, 1996; accepted for publication May 21, 1996.

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Abbreviations: ERE, estrogen response element; RT-PCR, reverse transcriptase polymerase chain reaction; SLE, systemic lupus erythematosus; SS-A/Ro, Sjögren syndrome antigen A or Ro antigen.

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containing 10% fetal bovine serum and 0.001% insulin. Cells were maintained by subculture twice a week.

Estradiol Stimulation Water-soluble 17-β-estradiol (Sigma, St. Louis, MO) solution was immediately prepared fresh for each experiment. Human keratinocytes were cultured to 50% confluence, and estradiol was added afterward to final concentrations of 10^{-9} M to 10^{-7} M. MCF-7 cells were grown in phenol red–free medium with 10% dextran-coated charcoal–treated bovine serum (Hyclone, Logan, UT) for 4 d, and estradiol was added to various final concentrations up to 10^{-7} M.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Human keratinocytes or MCF-7 cells were harvested from culture flasks using trypsin, and total cellular RNA was purified using UltrospecRNA isolation reagents (Biotecx, Houston, TX). Specific mRNA was analyzed using a simplified RT-PCR method described by Goblet *et al* (1989). Total RNA (0.1–1.0 μg) and 0.5 μM primers (0.5 μl each) were heated to 70°C for 10 min and then quickly chilled on ice. Remaining components including 1.25 U of *Taq* polymerase (Gibco BRL, Gaithersburg, MD), 100 U of SuperScript II RNase H-reverse transcriptase (Gibco BRL), 20 U of RNase inhibitor (Promega, Madison, WI), and 0.25 μl of 10 mM dNTPs; 2.5 μl of 10 × PCR buffer containing 500 mM KCl, 100 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, and 0.1% gelatin was added to a final total volume of 25 μl. For RT-PCR detection of the 52-kDa SS-A/Ro mRNA, the sense primer 5′-AAGCTCCAGGTGGCATTAG-3′ and anti-sense primer 5′-CAGAGTTCATGGGGAAAAGA-3′ were designed based on our published sequence (GenBank accession M35041), with expected PCR products of 1099 and 868 bp for 52α and 52β, respectively (Chan *et al*, 1995). For the 60-kDa SS-A/Ro, the sense primer 5′-TCAAGAAGGCAGAACCACAAAG-3′ and anti-sense primer 5′-GGAGATTAGCTGGGAAGTAGCTG-3′ were designed from the sequence reported (GenBank accession J04137), yielding a PCR product of 1458 bp corresponding to the predominant splicing product of 60-kDa SS-A/Ro (60α) (Chan, 1994; Chan and Buyon, 1994). For the detection of 60β, the anti-sense primer was substituted by primer 5′-ACAGTGTCCACCTGCACTCC-3′, derived from the 3′ end of the alternative cDNA described by Ben-Chetrit *et al* (1989) (GenBank accession M25077); the predicted RT-PCR product was 1360 bp. All reaction components were mixed in a single 0.5-ml microfuge tube before thermal cycling. The RT-PCR program consisted of a reverse transcription step (50°C for 1 h) and a denaturing step (94°C for 3 min), followed by 30 cycles of PCR (94°C for 5 s, 55°C for 5 s, and 72°C for 1 min). RT-PCR products were analyzed by agarose gel electrophoresis. Positive control plasmids encoding the respective SS-A/Ro cDNAs and primer sets were run with the RT step omitted for direct comparison to ensure that the RT-PCR products were accurate representations of each SS-A/Ro. The mRNA of the housekeeping gene coding for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as an internal control (100 ng total RNA/reaction). The differences in mRNA expression were either quantified by densitometry or estimated by comparison of results using serially diluted samples.

Western Blot Nuclear extracts were harvested from cells using lysis buffer (100 mM Tris-HCl, 150 mM NaCl, and 0.5% Nonidet P-40) (Chan and Pollard, 1992). The protein concentration of the extract was determined by Bio-Rad protein assay (Bio-Rad, Richmond, CA). Equivalent amounts (40 μg) of protein extracts were separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by electrotransfer to nitrocellulose paper. The unoccupied sites on the nitrocellulose were blocked with 5% nonfat milk in phosphate-buffered saline for 30 min. A human prototype anti–SS-A/Ro serum Ge (1:100) that recognizes both 52-kDa and 60-kDa SS-A/Ro proteins (Chan and Buyon, 1994) was incubated with the nitrocellulose for 1 h. After phosphate-buffered saline washes, the nitrocellulose was probed for 1 h with horseradish peroxidase–conjugated anti-human IgG diluted 1:2000 in milk–phosphate-buffered saline. Reactivities to the SS-A/Ro proteins were detected by the chemiluminescence method using ECL Western blotting reagents (Amersham Life Science, Arlington Heights, IL) as per the manufacturer's instructions.

Nucleotide Sequence Analysis The FINDPATTERNS program of the Genetics Computer Group Sequence Analysis Software Package (Department of Genetics, University of Wisconsin) was used to search for consensus sequences for the estrogen response element (ERE) (Burch *et al*, 1988; Klein-Hitpass *et al*, 1988) in the genes coding for 52-kDa and 60-kDa SS-A/Ro. The complete gene sequence of the 52-kDa SS-A/Ro (13,270 nucleotides, GenBank accession U01882; Chan *et al*, 1995) including more than 3 kb of 5′ upstream sequence was used for the analysis of ERE. For the 60-kDa SS-A/Ro, the search for putative ERE was restricted to the 5′ 12-kb sequence including exons 1 and 2 alone (Chan *et al*, in preparation). The initial search was performed with FINDPATTERNS using the "consensus ERE" sequence GGTCAnnnTGACC (Klein-Hitpass *et al*, 1988). When a perfect match was not detected, the sequence was reanalyzed by allowing a one- or two-nucleotide mismatch in the consensus ERE. The search was also extended to include other ERE sequences with perfect half-site TGACC and adjacent transcriptional elements such as Sp1 (Dubik and Shiu, 1992).

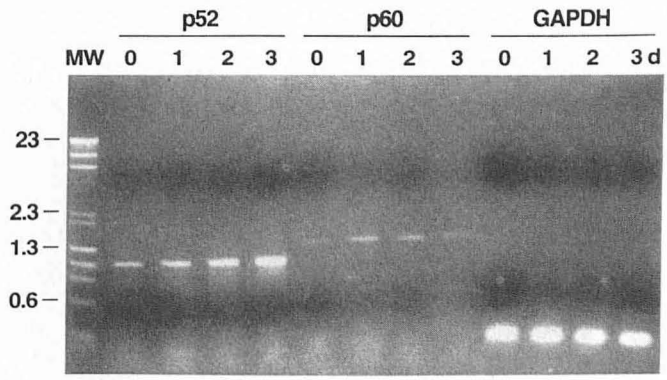

Figure 1. Increases in mRNA levels for 52-kDa and 60-kDa SS-A/Ro in human keratinocytes incubated with estradiol. Human keratinocytes were incubated with 10^{-7} M estradiol for 0, 1, 2, or 3 d. Total RNA samples of 0.3 μg each were analyzed by RT-PCR. Results showed a steady increase for the 52-kDa SS-A/Ro signal, whereas the 60-kDa SS-A/Ro signal peaked at day 1. No change in signal was detected for GAPDH mRNA using a 30-fold lower amount of total RNA.

RESULTS

Estradiol-Dependent SS-A/Ro mRNA Expression in Human Keratinocytes The initial rationale for examining the effect of estradiol on the expression of SS-A/Ro in keratinocytes was to determine whether the reported changes in cell surface expression (Furukawa *et al*, 1988, 1991) might be the result of alternative mRNA splicing in the SS-A/Ro genes described in our laboratory (Chan, 1994; Chan and Buyon, 1994; Chan *et al*, 1995). Based on previous reports (Furukawa *et al*, 1988, 1991), the first set of experiments was performed using human keratinocytes with 10^{-7} M estradiol incubated for 0, 1, 2, or 3 d **(Fig 1)**. The full-length 52-kDa SS-A/Ro, 52α, was detected as a 1.1-kb band, and the alternative spliced form 52β described in human fetal heart (Chan *et al*, 1995) was not observed. The predominant form of 60-kDa SS-A/Ro, 60α, was detected as a 1.45-kb band as predicted from the design of the RT-PCR primers. The alternative form 60β was not detected. **Figure 1** shows that both 52-kDa and 60-kDa SS-A/Ro mRNA levels increased after estradiol stimulation. The increase for the 52-kDa SS-A/Ro signal was gradual and proportional to the time of estradiol incubation, whereas the signal for 60-kDa SS-A/Ro reached a peak at day 1 and decreased gradually thereafter. The maximum increases of 52-kDa and 60-kDa protein mRNAs due to estradiol stimulation were 4–5–fold compared with untreated cells. Shorter incubation of estradiol (1 and 2 h) had no effect on both 52-kDa and 60-kDa SS-A/Ro mRNA expression (data not shown).

Figure 2 shows the response of SS-A/Ro mRNA expression in human keratinocytes stimulated by different concentrations of estradiol. Little or no increase in both SS-A/Ro forms was detected at 10^{-9} M compared with controls without estradiol. At 10^{-8} M, approximately 3-fold increases were detected for the 60-kDa SS-A/Ro, whereas a signal approximately 5-fold higher for the 52-kDa SS-A/Ro was observed. At 10^{-7} M estradiol, significant increases were detected compared with no estradiol, but signals were either the same (60-kDa SS-A/Ro) or lower (52-kDa SS-A/Ro) than at 10^{-8} M. Estradiol at 10^{-7} M was reported to be the optimal concentration to elevate SS-A/Ro protein expression in the human keratinocyte membranes (Furukawa *et al*, 1988).

It was interesting that three of the five batches of human keratinocytes purchased from Clonetics showed little or no effect of

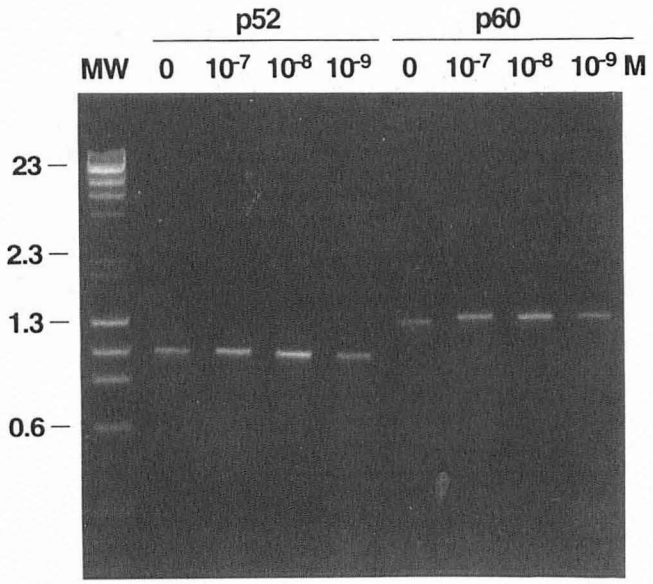

Figure 2. Analysis of SS-A/Ro mRNA expression. Human keratinocytes were incubated with estradiol at 0, 10^{-9}, 10^{-8}, or 10^{-7} M for 24 h.

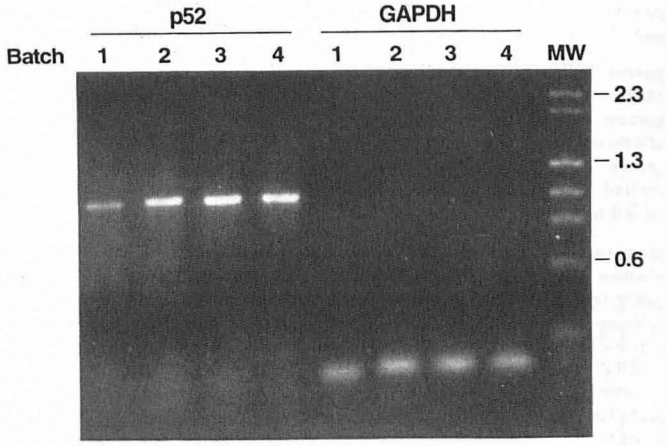

Figure 3. RT-PCR analysis of 52-kDa SS-A/Ro (0.2 μg total RNA/reaction). Analysis showed very low basal expression in batch 1 keratinocytes compared with batches 2, 3, and 4, whereas the levels for GAPDH were approximately the same (10 ng total RNA/reaction).

estradiol with respect to increased SS-A/Ro mRNA expression. **Figure 3** shows the basal level of 52-kDa SS-A/Ro from one of the two batches of keratinocytes (batch 1) that gave strong responses to estradiol at 10^{-8} to 10^{-7} M, compared with those from the three batches of cells (batches 2, 3, and 4) that showed little or no response. The high basal expression of SS-A/Ro in batches 2, 3, and 4 of human keratinocytes might be related to the failure to show a further increase in response to estradiol stimulation.

Estradiol-Dependent Expression of SS-A/Ro in the Human Breast Cancer Cell Line MCF-7 MCF-7 cells have estrogen receptors and are used extensively for the detection of estrogenic compounds (Rajendran *et al*, 1987; Soto *et al*, 1994; vom Saal *et al*, 1995). By determining whether estradiol has a similar effect on SS-A/Ro expression in MCF-7, it is possible to obtain supportive results independent of the variable basal expression observed in different batches of human keratinocytes. Because phenol red and fetal bovine serum used in tissue culture media have been shown to have estrogenic effects, MCF-7 cells were first cultured in phenol red–free medium containing 10% dextran-coated charcoal–depleted fetal bovine serum for 4 d. Estradiol (10^{-7} M) was then added to the cultures, and cells were harvested at various times up to 2 d. **Figures 4** and **5** show the corresponding changes in mRNA and protein levels for both 52-kDa and 60-kDa SS-A/Ro. The cellular mRNA and protein levels of both 52-kDa and 60-kDa SS-A/Ro decreased after 4-d depletion (*lane 0*, **Figs 4*A*,*B*, 5**) compared with control cultured cells without depletion (*Ctrl lane*, **Figs 4*A*,*B*, 5**); the latter cells were cultured in normal culture medium containing phenol red and fetal bovine serum. For cells cultured in the depleted medium, the 52-kDa SS-A/Ro mRNA clearly increased after 1 d of estradiol incubation **(Fig 4*A*)**, but the corresponding increase in protein level was more apparent at day 2 **(Fig 5)**. Although the RT-PCR signals for the 60-kDa SS-A/Ro were always weaker than those for the 52-kDa SS-A/Ro, the 60-kDa protein as detected with the prototype serum Ge showed a strong peak signal at 12 h (*0.5 d*, **Fig 5**). The peak protein expression of 60-kDa SS-A/Ro correlated well with the peak mRNA signal observed at 8 h **(Fig 4*B*)**. Interestingly, the mRNA and protein levels of 60-kDa SS-A/Ro returned to those at time 0 when estradiol was present for 24 h or longer **(Figs 4*B*, 5)**.

Putative ERE in Human Genes Encoding 52-kDa and 60-kDa SS-A/Ro The regulation of gene expression in cells by estrogen involves the interaction between estrogen receptor and EREs. A consensus ERE was described as a perfect palindrome with the sequence GGTCAnnnTGACC (Burch *et al*, 1988; Klein-Hitpass *et al*, 1988). Structure-function studies, however, showed that only the TGACC half site and its immediate flanking dinucleotides are required for estrogen receptor binding (Shupnik and Rosenzweig, 1991). Using the FINDPATTERNS program, we identified the sequence GGT*T*AttcTGACC, with one mismatch to the consensus ERE, approximately 1.5 kb upstream of the transcriptional start sites in the gene encoding 52-kDa SS-A/Ro **(Fig 6*A*)**. In the 5′ 12-kb sequence of the human gene encoding 60-kDa SS-A/Ro, a consensus ERE with either a one- or two-nucleotide mismatch was not detected. Further analysis showed that an ERE similar to that of c-*myc* (Dubik and Shiu, 1992) was detected, and this was an ERE half site separated by 11 nucleotides from an Sp1 transcriptional element (GGCGGG) **(Fig 6*B*)**.

DISCUSSION

Previous studies (Furukawa *et al*, 1988, 1991) have shown that estradiol could change the pattern of expression of SS-A/Ro, although the molecular forms of SS-A/Ro antigens were not defined. Our results extend the previous findings (Furukawa *et al*, 1988, 1991) and show stimulation of both 52-kDa and 60-kDa mRNA expression in cultured human keratinocytes that had low basal expression of SS-A/Ro. Unresponsive keratinocytes had higher basal levels of SS-A/Ro, suggesting that the cells were already stimulated by estrogenic compounds during isolation or that some individuals might already have maximal expression of SS-A/Ro in their keratinocytes. The latter point suggests that there may be variable expression of SS-A/Ro in different individuals. This view is consistent with the recent finding by Niimi *et al* (1995) of marked variations in the expression of both 52-kDa and 60-kDa SS-A/Ro antigens in the skin of different individuals, although the mechanism for these differences remains unknown. The enhanced expression of both SS-A/Ro antigens was also observed in the human breast cancer cell line MCF-7. It was further shown that culturing MCF-7 under hormone-deprived conditions reduced the levels of both forms of SS-A/Ro, and the addition of estradiol led to increases in SS-A/Ro expression. In addition, genomic DNA analysis showed that both genes encoding 52-kDa and 60-kDa SS-A/Ro have putative EREs. Therefore, we hypothesize that SS-A/Ro antigen expression is regulated by estrogen via intracellular estrogen receptors that interact directly with the putative EREs of the SS-A/Ro genes and initiate new transcription. Because anti-SS-A/Ro antibodies are predominant in females with autoimmune diseases such as SLE and Sjögren syndrome, the higher

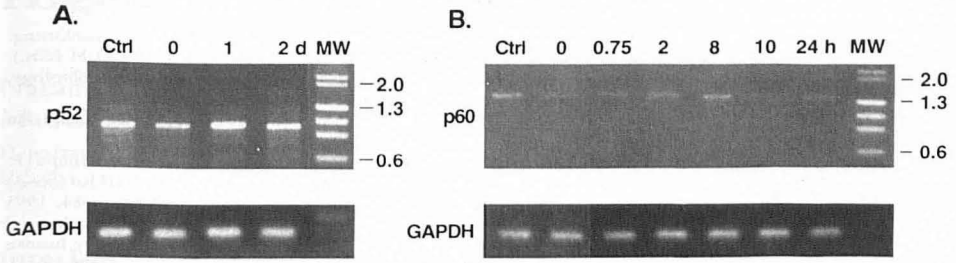

Figure 4. RT-PCR analysis of 52-kDa (*A*) and 60-kDa (*B*) SS-A/Ro mRNAs in MCF-7 cells first depleted of hormones and subsequently incubated with estradiol. MCF-7 cells were incubated in phenol red–free medium containing 10% dextran-coated charcoal–treated fetal bovine serum for 4 d. Estradiol (10^{-7} M) was added, and cells were harvested at different times thereafter. Total RNA of 0.2 μg each was used for RT-PCR to detect 52-kDa and 60-kDa SS-A/Ro mRNAs, and 10 ng of RNA was used for the GAPDH assay. Untreated control MCF-7 cells (*Ctrl*) were cultured in normal medium and therefore represent cells exposed to the estrogenic activity of phenol red and endogenous estradiol present in the fetal bovine serum of normal culture medium.

expression of SS-A/Ro antigens as a result of estrogenic influence may participate in sustaining or even triggering autoantibody production.

The estradiol-dependent expression of SS-A/Ro in cultured human keratinocytes required an estradiol concentration of 10^{-8} M or higher; no significant changes in SS-A/Ro mRNA were detected at 10^{-9} M estradiol. Our findings may be of physiologic significance, as concentrations of 10^{-8} M and 10^{-7} M estradiol are approximately equivalent to serum levels observed in late pregnancy (Levitz and Young, 1977). Therefore, enhanced levels of estradiol *in vivo* may trigger high levels of SS-A/Ro antigen expression. A recent article by Dörner *et al* (1995) reported that there are significant increases in both maternal and fetal anti–52-kDa SS-A/Ro and anti–SS-B/La antibodies during pregnancy in neonatal lupus.

The 52-kDa and 60-kDa SS-A/Ro had different expression kinetics in response to estradiol. For both keratinocytes and MCF-7 cells, the estradiol-associated increases for the 52-kDa protein were gradual and continued beyond day 1 of estradiol exposure. In contrast, the increases for the 60-kDa SS-A/Ro were short-lived, peaked between 8 and 24 h of estradiol incubation, and dropped gradually to background levels. This result suggests that the increase in SS-A/Ro expression was not due to an overall increase in transcription. It has been reported that the proliferative response associated with estradiol can be observed at 10^{-11} M (Krishnan *et al*, 1993). Because the results showed little or no change in SS-A/Ro levels until the concentration of estradiol was 10^{-8} M or higher, we can conclude that the elevated SS-A/Ro responses were not directly related to proliferative activity alone.

Two different types of ERE were proposed for the 52-kDa and 60-kDa SS-A/Ro genes, although it is clear that there are no experimental data to support that these two EREs are functional elements either *in vivo* or *in vitro*. The majority of EREs identified to date are located in the 5′ flanking region of target genes within 2 kb upstream of the promoter and have the imperfect palindrome structure GGTCAnnnTGACC (Klein-Hitpass *et al*, 1988). Furthermore, the second half of the consensus ERE (TGACC) seems to be more critical for estrogen receptor interaction in some cases (Hyder

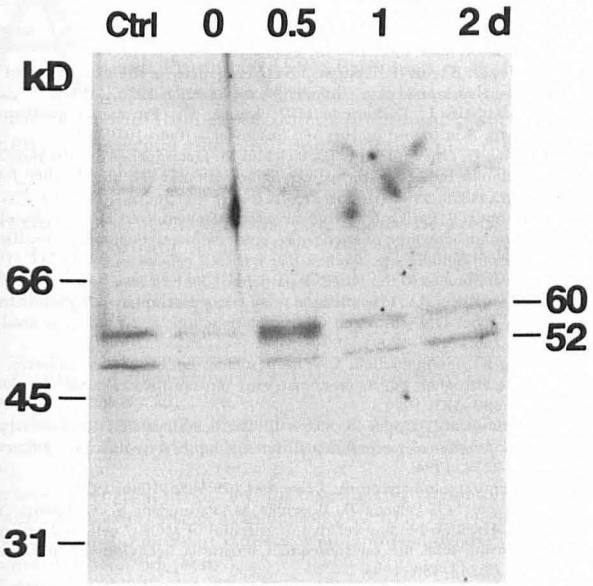

Figure 5. Western blot analysis of 52-kDa and 60-kDa SS-A/Ro proteins in MCF-7 cells first depleted of hormones and subsequently incubated with estradiol. As in **Fig 4**, MCF-7 cells were cultured in the hormone-depleted medium for 4 d, and cells were harvested 0, 0.5, 1, or 2 d after the addition of estradiol. Control MCF-7 cells were incubated in normal RPMI-1640 medium and represent cells exposed to the estrogenic activity of phenol red and estradiol present in the normal culture medium.

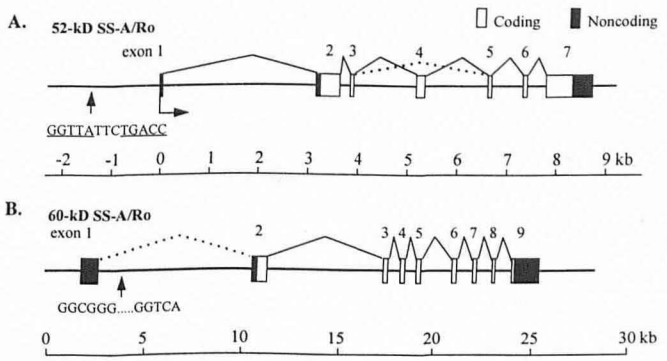

Figure 6. Putative EREs in human 52-kDa and 60-kDa SS-A/Ro genes. *A*) The 52-kDa SS-A/Ro gene (accession no. U01882) has a total of seven exons spread over 9 kb of DNA (Chan *et al*, 1995). Exon 1 contains a 5′ noncoding region that was detected in our original cDNA reported in 1991 (Chan *et al*, 1991). The translation initiation codon is located 3 kb downstream in exon 2, which also encodes the zinc finger domain. The rest of the coding region for the complete 52-kDa protein (52α) is located in exons 3 to 7. Exon 4 encodes the leucine zipper domain, which is one of the two coiled-coil regions. Exon 7 is the longest and encodes the rfp-like domain and the 3′-noncoding region. An alternative form 52β is detected resulting from the splicing of exon 3 to exon 5, skipping exon 4 (Chan *et al*, 1995). *B*) The 60-kDa SS-A/Ro gene has at least nine exons spanning a 30-kb region. Like the 52-kDa SS-A/Ro gene, exon 1 for the 60-kDa SS-A/Ro gene contains only noncoding regions, and the coding regions are located in exons 2–9. Exon 2 contains the RNA-binding domain.

et al, 1995). The putative ERE of 52-kDa protein, with only one mismatch, is potentially functional because it is located upstream of the promoter and within the range observed in other genes with known functional EREs (Shupnik and Rosenzweig, 1991). For the 60-kDa SS-A/Ro gene, the ERE shown had an Sp1 site (GGCGGG) flanking a second-half ERE consensus (GGTCA). Similar combinations of an Sp1 site flanking a half ERE site have been shown to be functional in the c-*myc* gene (Dubik and Shiu, 1992) and in the rat creatine kinase B gene (Pentecost *et al*, 1990). It is hypothesized that a weak interaction of the estrogen–estrogen receptor complex with the ERE half site is stabilized by an interaction with an adjacent bound Sp1 or Sp1-like factor (Dubik and Shiu, 1992). The fact that the putative ERE of the 60-kDa SS-A/Ro gene is located in intron 1 rather than the 5′ flanking region does not diminish its functional potential, as some functional EREs have been detected in exon (Hyder *et al*, 1995) and 3′-noncoding regions (Hyder *et al*, 1992).

Although SS-A/Ro proteins are localized to the nucleus and cytoplasm (Chan and Buyon, 1994), cell surface expression of SS-A/Ro has been described under certain conditions, including estradiol stimulation (Furukawa *et al*, 1988), tumor necrosis factor–α induction (Dörner *et al*, 1996), ultraviolet B exposure (Furukawa *et al*, 1990), and ultraviolet B plus cytomegalovirus infection (Zhu, 1996). It is unclear whether the cell surface expression of SS-A/Ro antigens described in these studies reflects the fact that cells were simply in an abnormal or dying state. For example, Casciola-Rosen *et al* (1994) showed that SS-A/Ro proteins clustered in surface blebs of apoptotic keratinocytes. Estradiol is known to enhance apoptosis, and it can also increase the expression of *bcl*-2, which is known to participate in the apoptotic pathway in MCF-7 cells (Wang and Phang, 1995). Antigens prominent in apoptosis may also influence various cellular and humoral aspects of autoimmune diseases (Tan, 1994). Translocation of antigen to the cell surface may be the direct or indirect result of elevated expression of SS-A/Ro antigens. Hence, the estradiol-dependent expression of SS-A/Ro described in this report may have significant implications, including skin manifestations in females with SLE.

We thank Dr. Robert L. Ochs for advice on culturing MCF-7 cells. This is publication 9837-MEM from The Scripps Research Institute. This work was supported by National Institutes of Health Grants AR-41803 and AR-07144.

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